



PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>C12N 15/11, 15/62</b>		A1	(11) International Publication Number: <b>WO 98/33903</b>
(21) International Application Number <b>PCT AU98 00057</b>		(43) International Publication Date: 6 August 1998 (06.08.98)	
(22) International Filing Date: 3 February 1998 (03.02.98)		(81) Designated States: AU, CA, CN, JP, NZ, SG, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(30) Priority Data: PO 4923 3 February 1997 (03.02.97) AU		(Published) <i>With international search report</i>	
(71) Applicant ( <i>for all designated States except US</i> ): COMMON-WEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION [AU AU], Limestone Avenue, Campbell, ACT 2601 (AU).			
(72) Inventor; and			
(73) Inventor/Applicant ( <i>for US only</i> ): MOLLOY, Peter, Laurence [AU AU]; 41 Bellevue Street, Chatswood, NSW 2067 (AU).			
(74) Agent: F.B. RICE & CO; 605 Darling Street, Balmain, NSW 2041 (AU).			

(54) Title: TISSUE SPECIFIC PROMOTERS

(57) Abstract

The present invention provides a genetic construct providing preferential expression in specific tissue of a coding sequence. The construct includes the following elements (1): a steroid responsive promoter from a tissue specific gene, (2) a coding sequence, and (3) an SV40 enhancer. The elements are arranged as follows: the steroid responsive promoter from the tissue specific gene being upstream of the coding sequence and the SV40 enhancer being 3' of the coding sequence, or the SV40 enhancer being upstream of the coding sequence and the steroid responsive promoter from the tissue specific gene being positioned between the SV40 enhancer and the coding sequence, or the steroid responsive promoter from the tissue specific gene being upstream of the coding sequence and the SV40 enhancer being positioned within an intron within the coding sequence. The promoter has a lowered level of steroid responsiveness in the construct than in its native state.

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BI	Belgium	GN	George	MK	The former Yugoslav	TM	Turkmenistan
BL	Burkina Faso	GR	Greece		Republic of Macedonia	TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's	NZ	New Zealand		
CM	Cameroon		Republic of Korea	PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

*Tissue Specific Promoters***FIELD OF THE INVENTION**

The present invention is concerned with combinations of prostate gene promoters with a general enhancer, the SV40 enhancer, giving rise to essentially androgen-independent promoters active in prostate cells which are androgen-independent in their growth and survival.

**BACKGROUND OF THE INVENTION**

A number of genes have been identified which are expressed predominantly in the prostate in males. Promoters and regulatory regions of a number of such genes have been studied, either using transfection techniques or by following gene expression in transgenic mice. Most of the genes identified as prostate-specific are androgen-inducible and this aspect of their function has been studied in some detail. Thus the importance of androgen response elements for induced expression and/or binding of androgen receptor have been characterised in the prostate specific antigen (PSA) (Cleutjens, Vaneekelen et al. 1996; Riegman, Vliestra et al. 1991), human glandular kallikrein (KLK2) (Murtha, Tindall et al. 1993), rat prostatic steroid binding proteins (PSBP) (Claessens, Rushmere et al. 1990; Rushmere, Parker et al. 1987), probasin (Pb) (Kasper, Rennie et al. 1994; Rennie, Bruchovsky et al. 1993) and prostatic acid phosphatase genes (Virkkunen, Hedberg et al. 1994) and in regulatory elements in the introns of the rat PSBP C3(1) gene (Celis, Claessens et al. 1993) and the rat 20-KDa androgen regulated protein (Ho, Marschke et al. 1993).

Elements involved in conferring prostate-specificity of expression per se, as distinct from androgen responsiveness, have not been well characterised, though tissue-specific factors binding to regions of the PSBP C3 gene promoter and 1st intron have been identified (Celis, Claessens et al. 1993; Zhang, Parker et al. 1990). The gene for rat PSBP C(3) with 4 kb upstream and 2 kb downstream flanking sequences is expressed tissue-specifically and with appropriate hormonal control in transgenic mice (Allison, Zhang et al. 1989). The use of a 5kb upstream region from the rat PSBP C3(1) gene to express the SV40 T-antigen could elicit prostate tumours, but expression was not highly restricted and other abnormalities were common (Maroulakou, Anver et al. 1994). Studies with transgenic mice

have established that regions of the probasin and PSBP C(3) genes can confer prostate specificity.

The PSA and probasin regulatory regions are the two most studied among prostate-expressed genes. It has been established that a 430 bp region upstream of the rat probasin gene is able to confer prostate specificity of expression on a reporter gene (Greenberg, Demayo et al. 1994; Matusik: WO9403594); when used to target expression of the SV40 T-antigen, prostate tumours develop specifically (Greenberg, Demayo et al. 1995). This expression is not totally specific but specificity is significantly improved by the inclusion of MAR (matrix attachment regions) from the chick lysozyme gene (Greenberg, Demayo et al. 1994). The 430 bp promoter region is strongly responsive to androgen induction and androgen response elements which bind the androgen receptor (AR) have been characterised (Claessens, Rushmere et al. 1990; Kasper, Rennie et al. 1994; Matusik, WO9403594; Rennie, Bruchovsky et al. 1993). A negative regulatory region between bases -426 and -286 has also been identified (Rennie, Bruchovsky et al. 1993).

The PSA upstream region (to -630 bp) also acts as a strongly androgen responsive promoter and androgen response elements have also been characterised. This region is not sufficient to direct tissue specific expression in transgenic mice, however. Use of the 630 bp human PSA promoter region to express an activated Ha-ras oncogene in transgenic mice led to the development of salivary gland and not prostate tumours (Schaffner, Barrios et al. 1995). An enhancer region has recently been identified in the region 4 to 5kb upstream of the transcription start site. The PSA enhancer has been shown to act as an androgen-inducible enhancer and in combination with the PSA promoter to display significant cell-type specificity (Henderson, WO9519434; Schuur, Henderson et al. 1996). Also Pang et al. have reported that the equivalent promoter region isolated from a prostate cancer patient contained 7 mutations compared to the published sequence and was highly active in the prostate cancer cell line LNCaP (Belldegrun and Pang, WO9614875; Pang, Taneja et al. 1995).

The viral SV40 enhancer was the first enhancer identified and it has been extensively characterised. It has the properties of enhancing expression from a variety of promoters whether it is upstream or downstream of the gene and is functional in either orientation. There are a limited number of reports where the SV40 enhancer has been used in

conjunction with a steroid-responsive promoter. For example Israel and Kaufman (Israel and Kaufman 1989) reported substantial dexamethasone induction of expression (up to 17 fold) in constructs which combined the SV40 enhancer and adenovirus major late promoter with different copy numbers of glucocorticoid response elements derived from the mouse mammary tumour virus LTR promoter/enhancer. Wynshaw-Boris et al. (Wynshaw-Boris, Short et al. 1986) found that the SV40 enhancer had little effect on basal level transcription or glucocorticoid-inducibility (about 6-fold) of the rat phosphoenolpyruvate carboxykinase gene. The 3 to 5-fold glucocorticoid induction mediated by intragenic sequences of the rat growth hormone gene was found to be equivalent in the presence or absence of the SV40 enhancer (Birnbaum and Baxter 1986). In another case it was observed that the glucocorticoid response region of the tyrosine amino transferase gene was able to confer 2 to 3 fold glucocorticoid inducibility on the SV40 enhancer/early promoter (Grange, Roux et al. 1989). These examples indicate that continued steroid responsiveness is normally seen when the SV40 enhancer is present in conjunction with a steroid-responsive promoter.

The present inventors have combined, in various constructs, the viral SV40 enhancer with the promoter regions of two prostate-expressed, androgen-induced genes - probasin and PSA. They have observed a substantial increase in the basal level of induction of the promoters without a corresponding increase in the androgen-induced level of activity. The promoter/enhancer combinations are thus now essentially androgen-independent, but maintain their specificity profile with respect to expression in prostate and non-prostate cells. Thus the probasin/SV40 enhancer combinations show substantial prostate specificity, while PSA/SV40 enhancer combinations are more promiscuous.

## SUMMARY OF THE INVENTION

Accordingly in a first aspect the present invention consists in a genetic construct providing preferential expression in specific tissue of a coding sequence, the construct including the following elements (1) a steroid responsive promoter from a tissue specific gene, (2) a coding sequence, and (3) an SV40 enhancer, the elements being arranged as follows:

the steroid responsive promoter from the tissue specific gene being upstream of the coding sequence and the SV40 enhancer being 3' of the coding sequence, or

5 the SV40 enhancer being upstream of the coding sequence and the steroid responsive promoter from the tissue specific gene being positioned between the SV40 enhancer and the coding sequence, or

the steroid responsive promoter from the tissue specific gene being upstream of the coding sequence and the SV40 enhancer being positioned within an intron within the coding sequence;

10 wherein the promoter has a lowered level of steroid responsiveness in the construct than in its native state.

In a second aspect the present invention consists in a genetic cassette comprising a steroid responsive promoter from a tissue specific gene and an SV40 enhancer and an insertion site into which a coding sequence can be 15 inserted, the insertion site being adjacent to and downstream of the promoter, wherein the promoter has a lowered level of steroid responsiveness in the cassette than in its native state.

In this aspect of the invention the SV40 enhancer may be upstream of the tissue specific promoter or *vice versa*.

20 In a preferred embodiment of the present invention the promoter from the tissue specific gene is androgen-responsive. It is also preferred that the tissue specific gene is prostate specific.

25 In further preferred embodiments the tissue specific promoter is the probasin promoter, in particular Pb430 or Pb286 as described herein. In another embodiment the promoter is the PSA promoter, in particular PSA630 as described herein.

In a third aspect the present invention consists in a vector including the genetic construct of the first aspect of the present invention or the 30 genetic cassette of the second aspect of the present invention.

It is presently preferred that the vector is human adenovirus Type 5 or ovine adenovirus.

35 In a further aspect the present invention provides methods of treatment of cancers, in particular, prostate, bladder and breast, involving gene therapy using the constructs of the present invention. The use of these constructs enables the use of androgen-ablation treatment in combination

with the expression of therapeutic genes in gene therapy for androgen-independent or androgen-dependent prostate cancer.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will 5 be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

#### DETAILED DESCRIPTION OF THE INVENTION

10 In order that the nature of the present invention may be more clearly understood preferred forms thereof will now be described with reference to the following non-limiting Examples.

#### FIGURE LEGENDS

- 15 **Figure 1A** shows a linearised map of the plasmid pCATSAT. Restriction enzyme sites H(Hind III), Sp(Sph I), P(PstI), S(SalI), X(XbaI) and BamHI are shown: amp and ori are the plasmid ampicillin resistance gene and origin of replication respectively. The chloramphenicol acetyl transferase (CAT) and serine acetyl transferase 20 genes are indicated as is the rous sarcoma virus promoter (RSV) and polyadenylation regions derived from the human growth hormone gene or SV40.
- 25 **Figure 1B** shows a number of promoter/enhancer constructs made in the pCATSAT vector. Promoter regions upstream of the CAT gene were derived from either the probasin (Pb430 or Pb286) or PSA (Psa630) genes. The SV40 enhancer is shown as a dark box, its orientation being indicated by the position of the NcoI (N) site.
- 30 **Figure 2** shows the sequence of SV40 enhancer. The BstYI sites used for cloning and the unique NcoI site are underlined.
- 35 **Figure 3** shows the transcriptional activity of constructs containing the Pb430 promoter in a number of cell types.
- Figure 4** shows the transcriptional activity of constructs containing the 286 base pair probasin promoter in a number of cell types.
- Figure 5** shows the transcriptional activity of constructs containing the 630 base pair PSA promoter in a number of cell types.

Figure 6 shows the recombinant adenovirus Ad5SVbPb430PNP. The cassette containing the SVbPb430 enhancer promoter, the E.coli purine nucleoside phosphorylase gene (PNP) and SV40 polyadenylation region (SVpolyA) is inserted into the deleted Ela/6 region of the human adenovirus type 5. Below, in brackets, is shown the equivalent cassette with the PSA630 base pair promoter included in the virus Ad5PSAPNP.

Figure 7A shows the effect of the combination of treatment with either Ad5SVbPb430PNP (SVPb) or Ad5PSAPNP in combination with 6-MPDR or PC3 cell viability. Viability was measured as metabolic activity relative to control, without virus or drug. MOI - multiplicity of infection.

Figure 7B shows the effect of virus plus drug treatment on MRC5 cells. Figure 7C shows the effect of virus plus drug treatment on HepG2 cells.

## EXAMPLES

### *Plasmid Constructs.*

The following plasmids combining the SV40 enhancer region in different positions and orientation with the promoter regions of the rat probasin and human PSA genes are diagrammed in Figure 1. All plasmids have been prepared using the base plasmid pCATSAT. This plasmid was derived from the plasmid pCAT-basic (Promega) by insertion into the BamHI site of a reference gene, serine acetyl transferase (SAT.) This reference reporter gene is under the control of the ubiquitously-expressed rous sarcoma virus promoter (RSV) and contains a 3' polyadenylation region derived from the human growth hormone gene. A BamHI site was regenerated as shown between the SV40 and human growth hormone 3' polyadenylation regions.

Probasin sequences from -426 (HindIII) or -286 to the SacI site at position +28 were isolated by PCR from rat genomic DNA and cloned into pCATSAT to produce the Pb430 and Pb286 constructs, respectively. For Pb430 the PCR fragment was cloned as a SacI fragment into the SacI site of pBluescriptSK+ using the SacI site at +28 and a site incorporated into the 5'

primer. It was then recloned as a HindIII to XbaI fragment into pCATSAT. The Pb286 PCR fragment was cloned into pBluescriptSK+ as an EcoRI to SpeI fragment using sites contained in the 5' and 3' primers respectively; it was then cloned as an HindIII to SpeI fragment into HindIII-XbaI cut pCATSAT. The PSA630 plasmid contained sequences 5' of the PSA transcription start site from EcoRI site (-630) to the HindIII site (+6) which were isolated from human genomic DNA by PCR amplification.

SV40 enhancer sequences were isolated as a BstYI fragment from the plasmid pCAT-enhancer (Promega). The sequence of the enhancer region is shown in Figure 2. The unique NcoI site is underlined. This fragment was subcloned into the BamHI sites of the plasmids Pb430CATSAT and PSA630CATSAT to produce the plasmids Pb430CATSATSVa and Pb430CATSATSVb and PSA630CATSATSVa and PSA630CATSATSVb respectively. The a and b orientations of the enhancer are distinguished by the position of the NcoI site as indicated in Figure 1. For placement of the enhancer upstream of the PB430 promoter the BstYI enhancer fragment was cloned into BamHI-cut pBluescriptSK+ (Stratagene), then re-cloned as a XbaI to KpnI fragment into pUC19. It was then excised as a HindIII fragment and cloned into the HindIII site of Pb430CATSAT to give the plasmids SVaPb430CATSAT and SVbPb430CATSAT. The SV40 enhancer was cloned in front of the Pb286 promoter in the pBluescriptSK+ plasmid using BamHI site incorporated in the 5' PCR primer. The two enhancer orientations in front of Pb286 were then re-cloned as HindIII-SpeI fragments into HindIII-XbaI cut pCATSAT to give plasmids SVaPb286CATSAT and SVbPb286CATSAT as shown in Figure 1.

#### Example 1:

A range of cell types were transfected with the Pb430CATSAT construct and plasmids containing the SV40 enhancer in either orientation either downstream of the CAT gene or immediately upstream of the probasin promoter.

#### Cell Types

Prostate tumour-derived cell lines used were LNCaP (obtained from Dr. L. Chung) and PC-3 (obtained from the ATCC). LNCaP cells are androgen-sensitive and express a mutated androgen receptor; PC-3, DU145 and TsuPr cells are androgen independent. Tumour cell lines derived from other tissues included the breast cancer line MCF-7 (obtained from the ATTC), the liver cell line HepG2 cells (from Dr. G. Schreiber), and the bladder cancer cell line BL13 (Russell, Wass et al. 1989). Human 293 embryonic kidney cells, adenovirus transformed, were obtained from Dr. F. Graham. The normal lung fibroblast line MRC5 and the chinese hamster ovary line CHO K1 were obtained from Dr. R. Holliday. LNCaP cells were maintained in T-medium (Thalmann, Sikes et al. 1996). PC-3, DU145 and TsuPr MCF7 and BL13 cells were maintained in RPMI 1640 medium containing 2mM glutamine, non-essential amino acids and 10% fetal bovine serum (FBS). HepG2 and MRC5 cells were maintained in Dulbecco's MEM containing 2 mM glutamine, 0.45% glucose and 10% FBS. CHO and 293 cells were maintained in MEM containing non-essential amino acids and 10% FBS.

#### *Transfections*

For transfection, cells were seeded at 30 to 50% confluence in 35mm dishes and transfected the following day using DOTAP (Boehringer) according to the manufacturer's protocols. Amounts of DNA and DOTAP were optimised for each cell type. Generally, 2.5 $\mu$ g of DNA mixed with 15 $\mu$ l of DOTAP was used to transfect one dish. Control transfections contained either plasmid (pBR322) DNA as a negative control or a standard mix of RSVCAT and RSVSAT plasmids as a positive control. Transfection of MRC5 cells was carried out using Lipofectamine (Gibco-BRL) according to the manufacturer's instructions. Transfections of DU145 and TsuPr cells were done using another cationic lipid transfection reagent CS067 and a similar protocol provided by Dr. T. Lockett (other transfection reagents such as Lipofectamine may also be used).

#### *CAT and SAT assays:*

At 44-48hr post-transfection cells were rinsed three times with PBS. and harvested by scraping into a 1.5ml centrifuge tube and brief centrifugation. Cell pellets were resuspended in 60 $\mu$ l of 0.1M Tris.HCl, pH7.5, containing 500 $\mu$ M Pefabloc protease inhibitor (Boehringer). Cells 5 were lysed by three cycles of freezing and thawing and debris was pelleted for 5min in a microfuge. Supernatant (20 $\mu$ l) was set aside for SAT assays and the remaining extract was heated at 65°C for 10 min. Debris was again pelleted and the supernatant used for CAT assays (Sleigh 1986). Non-heated extract was used for assays of serine acetyl transferase activity. Reaction 10 mixtures (20 $\mu$ l) comprised 2 or 4 $\mu$ l of cell extract, 1mM acetyl coenzyme A and 200 $\mu$ M serine containing 0.1 $\mu$ Ci <sup>14</sup>C-serine (Amersham, 50 mCi/ml, 150 mCi/mmol). Aliquots were removed between 20 and 120 min and reactions were stopped by heating at 95°C for 3 min. Reactions products were 15 separated by thin layer chromatography and subjected to phosphor image analysis (Molecular Dynamics). The extent of conversion of serine to acetylated serine was determined using ImageQuant software. The ratio of RSVCAT to RSVSAT in the control transfection in each experiment was used to normalise promoter activities relative to the RSV promoter.

20 *Promoter Activities*

Activities of the constructs containing the Pb430 promoter and different arrangements of the SV40 enhancer in different cell types are shown in Figure 2. The promoter alone shows low activity in the LNCaP 25 prostate cell line and negligible activity in the PC-3 line which lacks an androgen receptor. Co-transfection of the androgen receptor into PC-3 cells leads to a strong stimulation of expression, indicating the critical androgen-dependence of the promoter. Among the non-prostate cell lines slight activity was seen in the MCF-7, breast, and HepG2, liver, cell lines. For all 30 four enhancer-containing constructs increased expression was seen in most cell types. The most dramatic increase was seen in the PC-3, DU145 and TsuPr prostate cell lines where expression ranged from 10 to 60% that of the RSV promoter. This high level expression is seen in the absence of co-transfection of the androgen receptor. Indeed, when the androgen receptor 35 was co-transfected activity was generally found to be lower in PC-3 cells, perhaps reflecting competition between binding factors or stimulatory

pathways. A similar effect was seen in the DU145 and TsuPr lines (data not shown). The Pb430SVa, SVaPb430 and SVbPb430 constructs showed 3.5 to 6 fold higher expression in the prostate PC-3 and DU145 cells than in any of the other cell types tested. Highest expression in non-prostate cell types was  
5 seen in the cell lines of secretory epithelial origin, the breast cancer cell line MCF-7 and the bladder cancer cell line BL13. It should be noted that the androgen receptor is expressed in the MCF-7 line. Expression in the other cell lines, 293 (kidney), MRC5 (lung fibroblast) and HepG2 (liver) was much lower, at least 20 fold, for example, for the SVbPb430 construct. Other  
10 experiments have shown that the Pb430 promoter can also be activated by AR gene co-transfection in most non-prostate cell lines. Similarly to their function in PC-3 cells the Pb430/SV40 enhancer combinations show no or much reduced requirement for AR gene co-transfection. Thus the probasin promoter/SV40 enhancer combinations provide for substantial specificity for  
15 expression in the PC-3 androgen-independent prostate cells relative to other cell types. The lack of requirement for the presence of androgen receptor for high level expression also provides for a significant improvement in utility compared to the probasin promoter alone, as these combinations will be active in androgen-independent cancer cells and can be used while  
20 continuing androgen ablation therapy designed to eliminate androgen-dependent tumour cells.

### Example 2

25 A range of cell types were transfected with the Pb286CATSAT construct and plasmids containing the SV40 enhancer in either orientation either immediately upstream of the Pb286 promoter.

Results of transfections are shown in Figure 4. As for the Pb430  
30 promoter, the Pb286 promoter is very poorly expressed in PC-3 cells, but is highly expressed when co-transfected with the androgen receptor. Again, combination of the Pb286 promoter with the SV40 enhancer are highly expressed in PC-3 cells in the absence of the androgen receptor. For the SVb enhancer orientation expression was 70% of that seen in the presence of the androgen receptor, while for the SVa orientation expression was reduced in  
35 the presence of the AR (similarly to the Pb430 constructs). While expression

was higher in PC-3 cells than in non-prostate cell types, the specificity of expression was less than that seen for the Pb430 constructs. The effect of the enhancer is thus similar to that seen for the Pb430 constructs in bypassing the requirement for androgen induction for expression from the promoter.

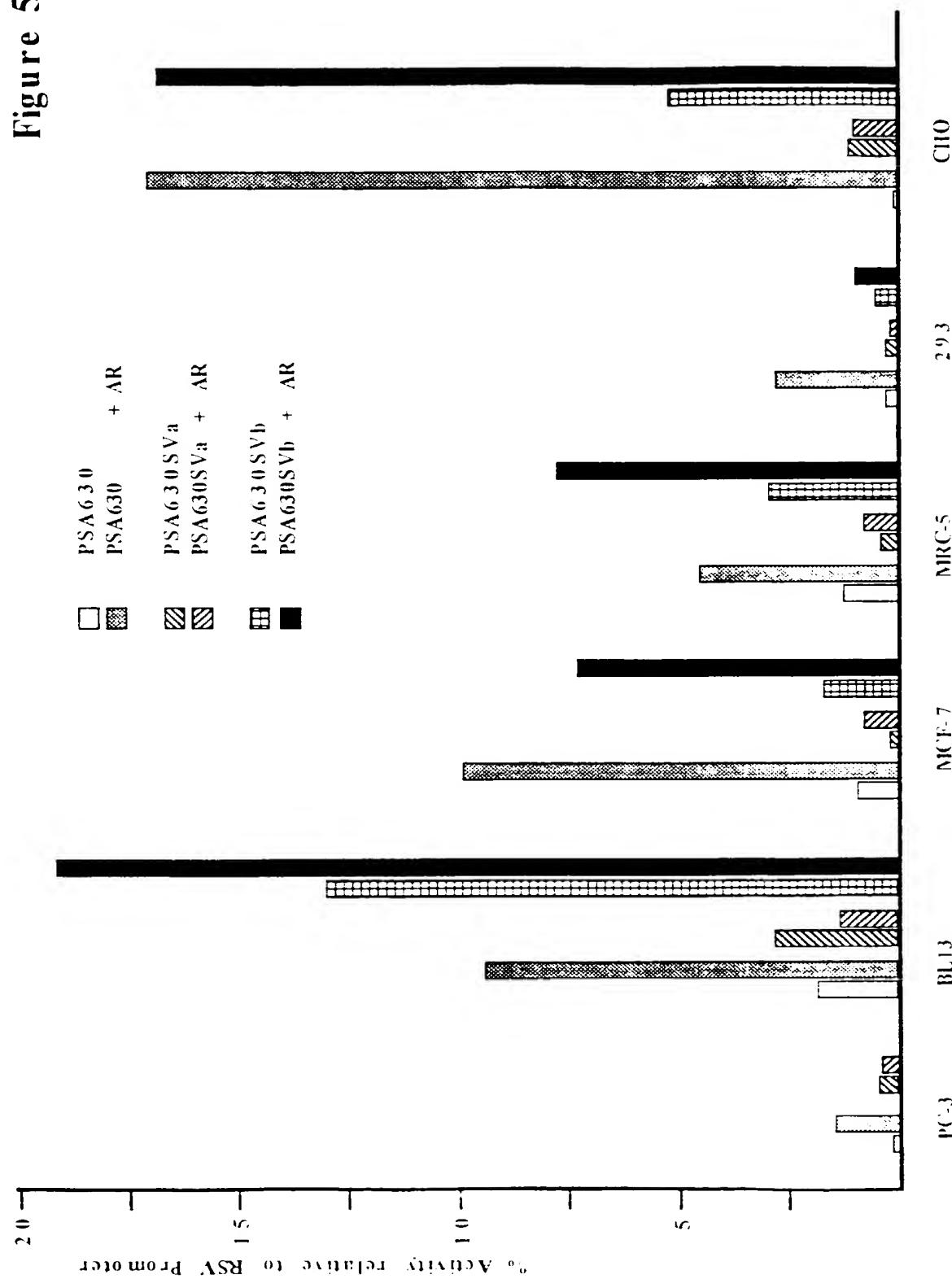
### Example 3.

Reduced AR requirement for expression from PSA promoter constructs incorporating the SV40 enhancer.

Expression from promoter constructs PSA630, PSA630SVa and PSA630SVb was examined in a number of cell lines following transfection alone or in combination with the AR gene. Results are shown in Figure 5. Expression from the PSA630 promoter was found to be significantly increased in the presence of AR in all cell types studied, though the level of induction varied considerably. Constructs containing the SV40 enhancer in either orientation 3' of the CAT gene showed substantially less enhancement of expression in the presence of AR in almost all cell types. The effect of AR ranged from three fold induction to two fold repression in different instances. The only exception was in MCF-7 cells where the AR enhanced expression by 5.8 and 4.3 fold for the SVa and SVb constructs respectively compared to tenfold in the absence of the enhancer. While these PSA630-based constructs show poor prostate specificity the effect of the SV40 enhancer on expression from this androgen-responsive promoter is similar to that on the Pb promoters in that expression becomes substantially androgen-independent.

### Example 4

The promoter cassette SVbPb430 was excised from pSVbPb430CATSAT as a SalI to XbaI fragment and blunt-ended. It was re-cloned in front of the E.coli purine nucleoside phosphorylase (PNP) gene in the plasmid pXCX3 PSAPNPase (Lockett et al. 1997) which had been cut with KpnI and HindIII, blunt-ended and re-ligated to remove the PSA promoter and subsequently cut with SpeI and blunt-ended. This was then used to construct, via recombination, a recombinant human Type 5

**Figure 5**

prostate-specific manner in both androgen-responsive and androgen-independent prostate cells and in the absence of androgens (as in cancer patients undergoing androgen-ablation treatment). This is of particular importance for example in the expression of therapeutic genes in gene therapy for androgen-independent prostate cancer.

As will be understood the constructs of the present invention will also be useful where patient has been treated surgically (castration) or with inhibitors of androgen synthesis or action. Specifically such androgen-blockade therapy can be continued without substantially diminishing the activity of these promoters.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

## REFERENCES

- Allison, J., Y.-L. Zhang and M. G. Parker. (1989). "Tissue-specific and  
5 hormonal regulation of the gene for rat prostatic steroid-binding protein in  
transgenic mice." Mol. Cell. Biol. 9: 2254-2257.
- Belldegrun, A. S. and S. Pang. "Nucleic acid contg. prostate specific antigen  
promoter useful for prodn. of heterologous protein, or for gene therapy of  
10 prostatic cancer." : Patent, WO9614875
- Birnbaum, M. J. and J. D. Baxter. (1986). "Glucocorticoids regulate the  
expression of a rat growth hormone gene lacking 5' flanking sequences." J.  
15 Biol. Chem. 261(1): 291-297.
- Celis, L., F. Claessens, B. Peeters, W. Heyns, G. Verhoeven and W.  
Rombauts. (1993). "Proteins interacting with an androgen-responsive unit in  
the C3(1) gene intron." Mol. Cell. Endocrinol. 94: 165-172.
- Claessens, F., N. K. Rushmere, P. Davies, L. Celis, B. Peeters and W. A.  
Rombauts. (1990). "Sequence-specific binding of androgen-receptor  
20 complexes to prostatic binding protein genes." Mol. Cell. Endocrinol. 74:  
203-212.
- Cleutjens, K. B. J. M., C. C. E. M. Vaneekelen, H. A. G. M. Vanderkorput, A.  
O. Brinkmann and J. Trapman. (1996). "Two androgen response regions  
cooperate in steroid hormone regulated activity of the prostate-specific  
25 antigen promoter." J Biol Chem. 271(11): 6379-6388.
- Grange, T., J. Roux, M. Fromont-Racine and R. Pictet. (1989). "Positive and  
negative regulation of a transfected chimeric tyrosine aminotransferase gene:  
effect of copy number." Exp. Cell Res. 180: 220-223.
- Greenberg, N. M., F. Demayo, M. J. Finegold, D. Medina, W. D. Tilley, J. O.  
30 Aspinall, G. R. Cunha, A. A. Donjacour, R. J. Matusik and J. M. Rosen.

- (1995). "Prostate cancer in a transgenic mouse." Proc Natl Acad Sci USA. 92(8): 3439-3443.
- 5 Greenberg, N. M., F. J. Demayo, P. C. Sheppard, R. Barrios, R. Lebovitz, M. Finegold, R. Angelopoulou, J. G. Dodd, M. L. Duckworth, J. M. Rosen and R. J. Matusik. (1994). "The rat probasin gene promoter directs hormonally and developmentally regulated expression of a heterologous gene specifically to the prostate in transgenic mice." Mol Endocrinol. 8(2): 230-239.
- 10 Henderson, D. R. Transcriptional regulator specific for cells expressing prostate specific antigen used to express toxins, immunostimulants or anti-sense cpds., for treatment and prevention of prostatic cancer or hypertrophy. Patent. WO9519434
- 15 Ho, K. C., K. B. Marschke, J. A. Tan, S. G. A. Power, E. M. Wilson and F. S. French. (1993). "A complex response element in intron-1 of the Androgen-Regulated 20-kDa protein gene displays cell Type-Dependent androgen receptor specificity." J Biol Chem. 268(36): 27226-27235.
- 20 Israel, D. I. and R. J. Kaufman. (1989). "Highly inducible expression vectors containing multiple GRE's in CHO cells overexpressing the glucocorticoid receptor." Nucleic Acids Res. 17(12): 4589-4604.
- Kasper, S., P. S. Rennie, N. Bruchovsky, P. C. Sheppard, H. Cheng, L. Lin, R.  
25 P. C. Shiu, R. Snoek and R. J. Matusik. (1994). "Cooperative binding of androgen receptors to two DNA sequences is required for androgen induction of the probasin gene." J. Biol. Chem. 269(50): 31763-31769.
- 30 Lockett LJ, Molloy PL, Russell PJ and Both GW. Relative efficiency of tumour cell killing *in vitro* by two enzyme-prodrug systems delivered by identical adenovirus vectors. Clinical Cancer Research. In press (1997)
- Maroulakou, I. G., M. Anver, L. Garrett and J. E. Green. (1994). "Prostate and mammary adenocarcinoma in transgenic mice carrying a rat c3(1) simian virus 40 large tumor antigen fusion gene." Proc Natl Acad Sci USA. 91(23): 11236-11240.

- Matusik, R. J. Isolated DNA mol. contg. an androgen responsive element comprising a 5'-flanking region of the rat probasin gene, used to generate transgenic animals and for human prostate cancer therapy. Patent.
- 5 WO9403594
- Murtha, P., D. J. Tindall and C. Y. F. Young. (1993). "Androgen induction of a human Prostate-Specific kallikrein, hKLK2 - characterization of an androgen response element in the 5' promoter region of the gene." Biochemistry. 32(25): 6459-6464.
- Pang, S., S. Taneja, K. Dardashti, P. Cohan, R. Kaboo, M. Sokoloff, C.-L. Tso, J. B. Dekernion and A. S. Belldegrun. (1995). "Prostate tissue specificity of the prostate-specific antigen promoter isolated from a patient with prostate cancer." Hum. Gene Ther. 6: 1417-1426.
- Rennie, P. S., N. Bruchovsky, K. J. Leco, P. C. Sheppard, S. A. McQueen, H. Cheng, R. Snoek, A. Hamel, M. E. Bock, B. S. MacDonald, B. E. Nickel, C. Chang, S. Liao, P. A. Cattini and R. J. Matusik. (1993). "Characterization of two *cis*-acting DNA elements involved in the regulation of the probasin gene." Molec. Endocrinol. 7(1): 23-36.
- Riegman, P. H. J., R. J. Vliestra, J. A. G. M. van der Korput, A. O. Brinkmann and J. Trapman. (1991). "The promoter of the prostate-specific antigen gene contains a functional androgen responsive element." Molec. Endocrinology. 5(12): 1921-1930.
- Rushinere, N. K., M. G. Parker and P. Davies. (1987). "Androgen-receptor binding regions of an androgen responsive gene." Mol. Cell. Endocrinol. 51: 259-265.
- Russell, P. J., J. Wass, R. Lukeis, O. M. Garson, M. Jelbart, E. J. Wills, J. Philips, J. Brown, N. Carrington, P. C. Vincent and D. Raghavan. (1989). "Characterization of cell lines derived from a multiply aneuploid human bladder transitional-cell carcinoma, UCRU-BL-13." Int. J. Cancer. 44: 276-285.

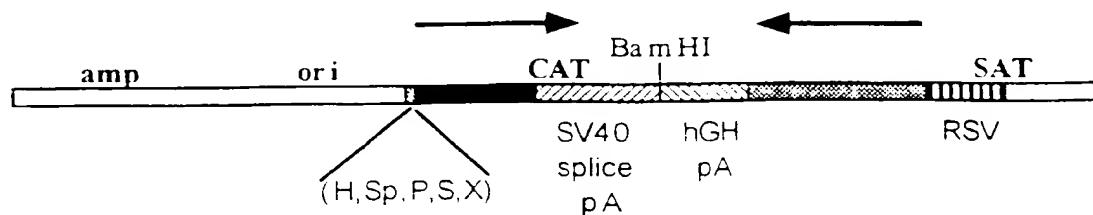
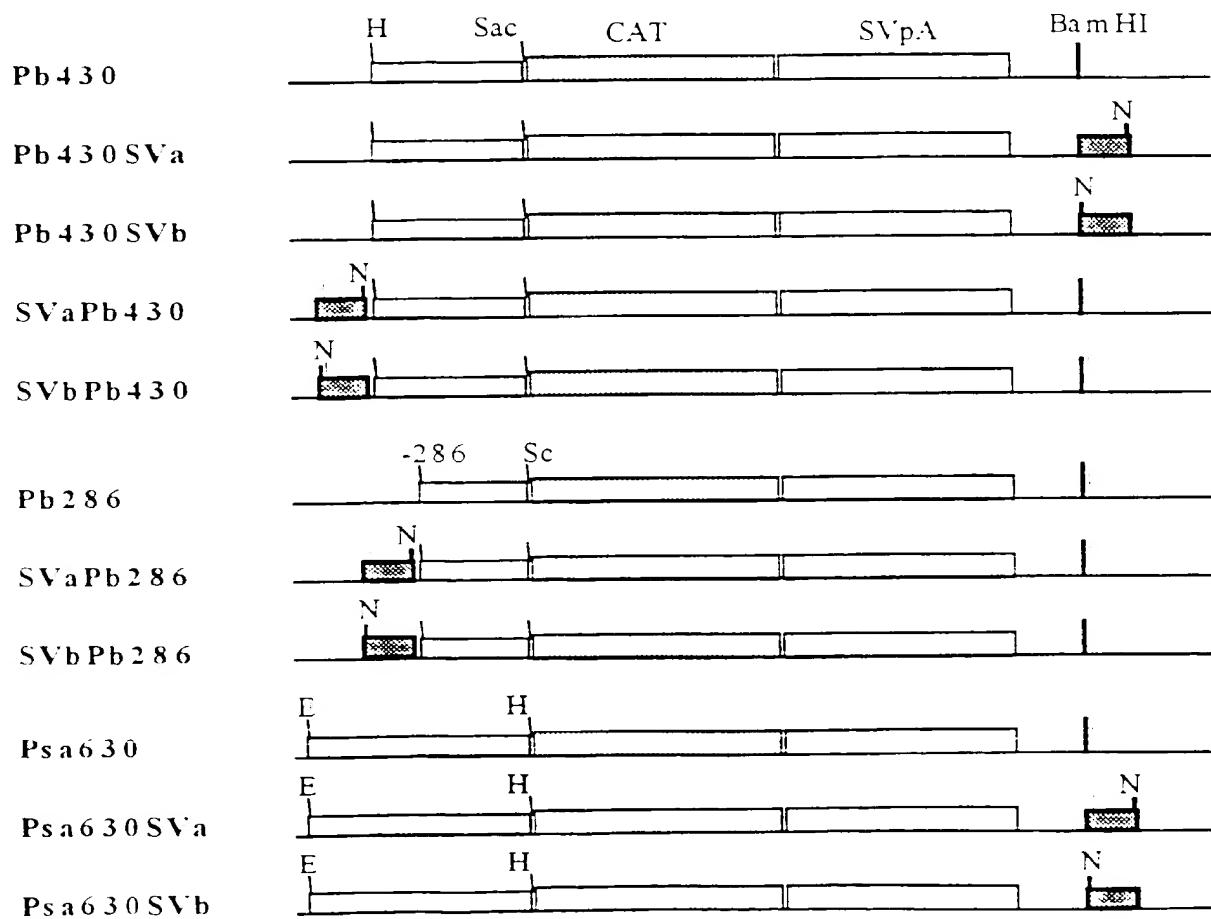
- Schaffner, D. L., R. Barrios, M. R. Shaker, S. Rajagopalan, S. L. Huang, D. J. Tindall, C. Y. F. Young, P. A. Overbeek, R. M. Lebovitz and M. W. Lieberman. (1995). "Transgenic mice carrying a PSArasT24 hybrid gene develop salivary gland and gastrointestinal tract neoplasms." *Lab Invest.* 72(3): 283-290.
- Schuur, E. R., G. A. Henderson, L. A. Kinetec, J. D. Miller, H. G. Lamparski and D. R. Henderson. (1996). "Prostate-specific antigen expression is regulated by an upstream enhancer." *J Biol Chem.* 271(12): 7043-7051.
- Sleigh, M. J. (1986). "A nonchromatographic assay for expression of the chloramphenicol acetyltransferase gene in eucaryotic cells." *Anal. Biochem.* 156(1): 251-256.
- Thalmann, G. N., R. A. Sikes, S.-M. Chang, D. A. Johnston, A. C. von Eschenbach and L. W. K. Chung. (1996). "Suramin-induced decrease in prostate-specific antigen expression with no effect on tumor growth in the LNCaP model of human prostate cancer." *J. Natl. Cancer Inst.* 88(12): 794-801.
- Virkkunen, P., P. Hedberg, J. J. Palvimo, E. Birr, K. Porvari, M. Ruokonen, P. Taavitsainen, O. A. Jänne and P. Vihko. (1994). "Structural comparison of human and rat prostate-specific acid phosphatase genes and their promoters: identification of putative androgen response elements." *Biochem. and Biophys. Res. Commun.* 202(1): 49-57.
- Wynshaw-Boris, A., J. M. Short, D. S. Loose and R. W. Hanson. (1986). "Characterization of the phosphoenolpyruvate carboxykinase (GTP) promoter-regulatory region. 1. Multiple hormone regulatory elements and the effects of enhancers." *J. Biol. Chem.* 261(21): 9714-9720.
- Zhang, Y.-L., M. G. Parker and O. Bakker. (1990). "Tissue-specific differences in the binding of nuclear proteins to a CCAAT motif in the promoter of the androgen-regulated C3 gene." *Molec. Endocrinol.* 4(8): 1219-1225.

## Claims:-

1. A genetic construct providing preferential expression in specific tissue of a coding sequence, the construct including the following elements (1) a steroid responsive promoter from a tissue specific gene, (2) a coding sequence, and (3) an SV40 enhancer, the elements being arranged as follows:
  - 5 the steroid responsive promoter from the tissue specific gene being upstream of the coding sequence and the SV40 enhancer being 3' of the coding sequence, or
  - 10 the SV40 enhancer being upstream of the coding sequence and the steroid responsive promoter from the tissue specific gene being positioned between the SV40 enhancer and the coding sequence, or
  - 15 the steroid responsive promoter from the tissue specific gene being upstream of the coding sequence and the SV40 enhancer being positioned within an intron within the coding sequence; wherein the promoter has a lowered level of steroid responsiveness in the construct than in its native state.
2. A genetic construct as claimed in claim 1 in which the steroid responsive promoter from the tissue specific gene is upstream of the coding sequence and the SV40 enhancer is 3' of the coding sequence.
3. A genetic construct as claimed in claim 1 in which the SV40 enhancer is upstream of the coding sequence and the steroid responsive promoter from the tissue specific gene is positioned between the SV40 enhancer and the coding sequence.
- 25 4. A genetic construct as claimed in claim 1 in which the steroid responsive promoter from the tissue specific gene is upstream of the coding sequence and the SV40 enhancer is positioned within an intron within the coding sequence.
5. A genetic construct as claimed in any one of claims 1 to 4 in which the steroid responsive promoter from the tissue specific gene is androgen-responsive.
- 30 6. A genetic construct as claimed in any one of claims 1 to 5 in which the tissue specific gene is prostate specific.
7. A genetic construct as claimed in any one of claims 1 to 6 in which the steroid responsive promoter from the tissue specific gene is the probasin promoter.

8. A genetic construct as claimed in claim 7 in which the promoter is Pb430 or Pb286.
9. A genetic construct as claimed in any one of claims 1 to 6 in which the steroid responsive promoter from the tissue specific gene is the PSA promoter.  
5
10. A genetic construct as claimed in claim 9 in which the promoter is PSA630.
11. A genetic cassette comprising a steroid responsive promoter from a tissue specific gene and an SV40 enhancer and an insertion site into which a coding sequence can be inserted, the insertion site being adjacent to and downstream of the promoter, wherein the promoter has a lowered level of steroid responsiveness in the cassette than in its native state.  
10
12. A genetic cassette as claimed in claim 11 in which the SV40 enhancer is upstream of the steroid responsive promoter of the tissue specific gene.
13. A genetic cassette as claimed in claim 11 in which the SV40 enhancer is downstream of the steroid responsive promoter of the tissue specific gene.  
15
14. A genetic cassette as claimed in any one of claims 11 to 13 in which the steroid responsive promoter of the tissue specific gene is androgen-responsive.
15. A genetic cassette as claimed in any one of claims 11 to 14 in which the tissue specific gene is prostate specific.  
20
16. A genetic cassette as claimed in any one of claims 11 to 15 in which the steroid responsive promoter of the tissue specific gene is the probasin promoter.
17. A genetic cassette as claimed in claim 16 in which the promoter is Pb430 or Pb286.  
25
18. A genetic cassette as claimed in any one of claims 11 to 15 in which the steroid responsive promoter of the tissue specific gene is the PSA promoter.
19. A genetic cassette as claimed in claim 18 in which the promoter is PSA630.  
30
20. A vector including the genetic construct as claimed in any one of claims 1 to 10 or the genetic cassette as claimed in any one of claims 11 to 19.
21. A vector as claimed in claim 20 in which the vector is human adenovirus Type 5.  
35

22. A vector as claimed in claim 20 in which the vector is ovine adenovirus.

**Figure 1****A. pCATSAT****B. Promoters/enhancers constructs**

**Figure 2.**

Sequence of SV40 Enhancer region

GGATCTGAAC CATGGAGCGG AGAATGGGCG GAACTGGGCG GAGTTAGGG

BstYI NcoI

CGGGATGGGC GGAGTTAGGG CGGGGACTAT GTTGTCTGAC TAATTGAGAT

GCATGCTTTG CATACTTCTG CCTGCTGGGG AGCCTGGGGA CTTTCCACAC

CTGGTTGCTG ACTAATTGAG ATGCATGCTT TGCATACTTC TGCCTGCTGG

GGAGCTGGG GACTTTCCAC ACCCTAACTG ACACACATTC CAAGGCAGAT

CC

BstYI

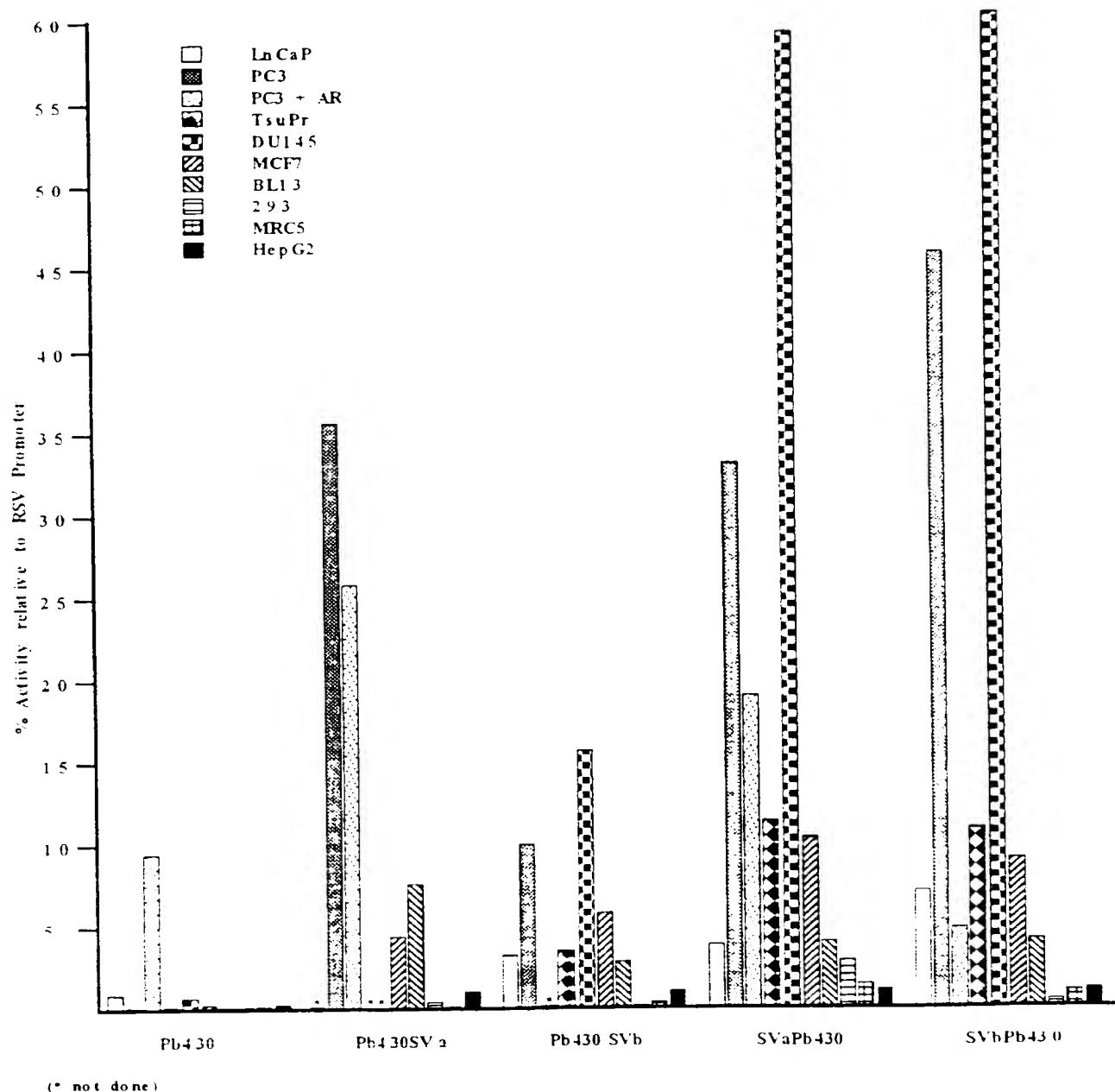
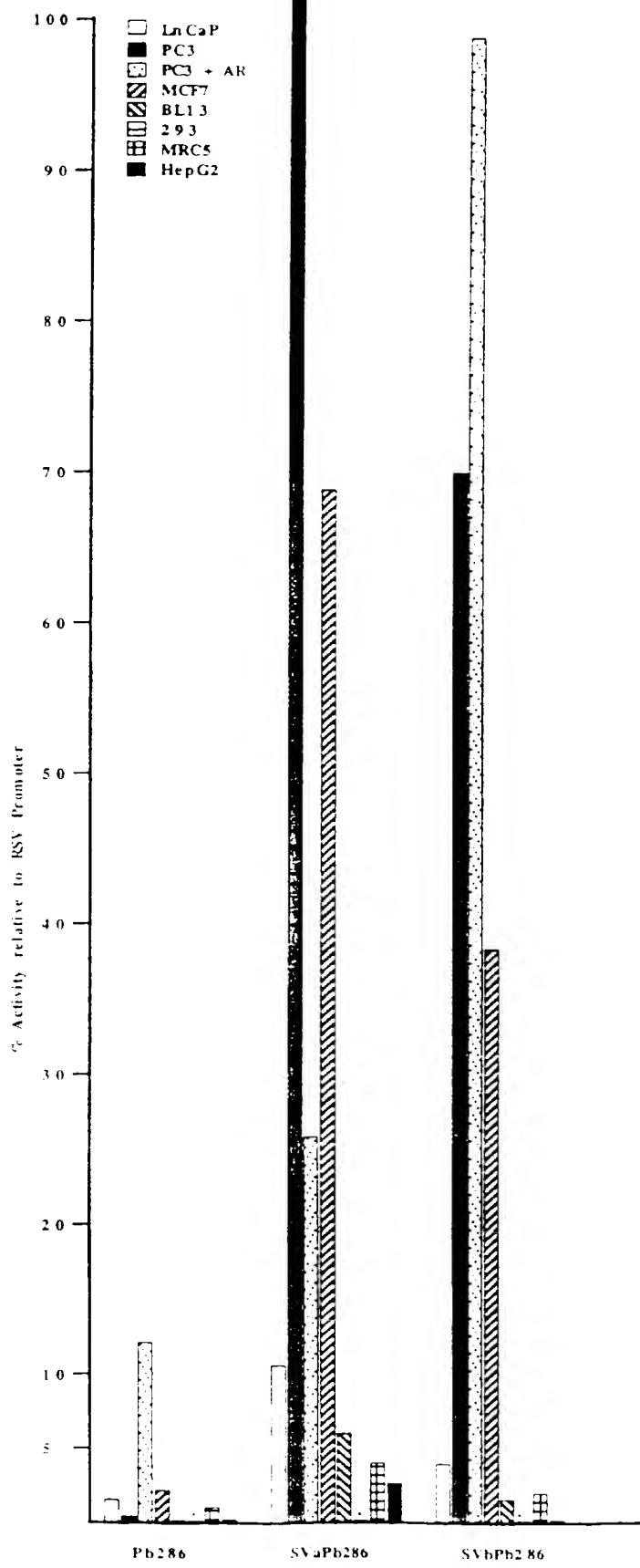
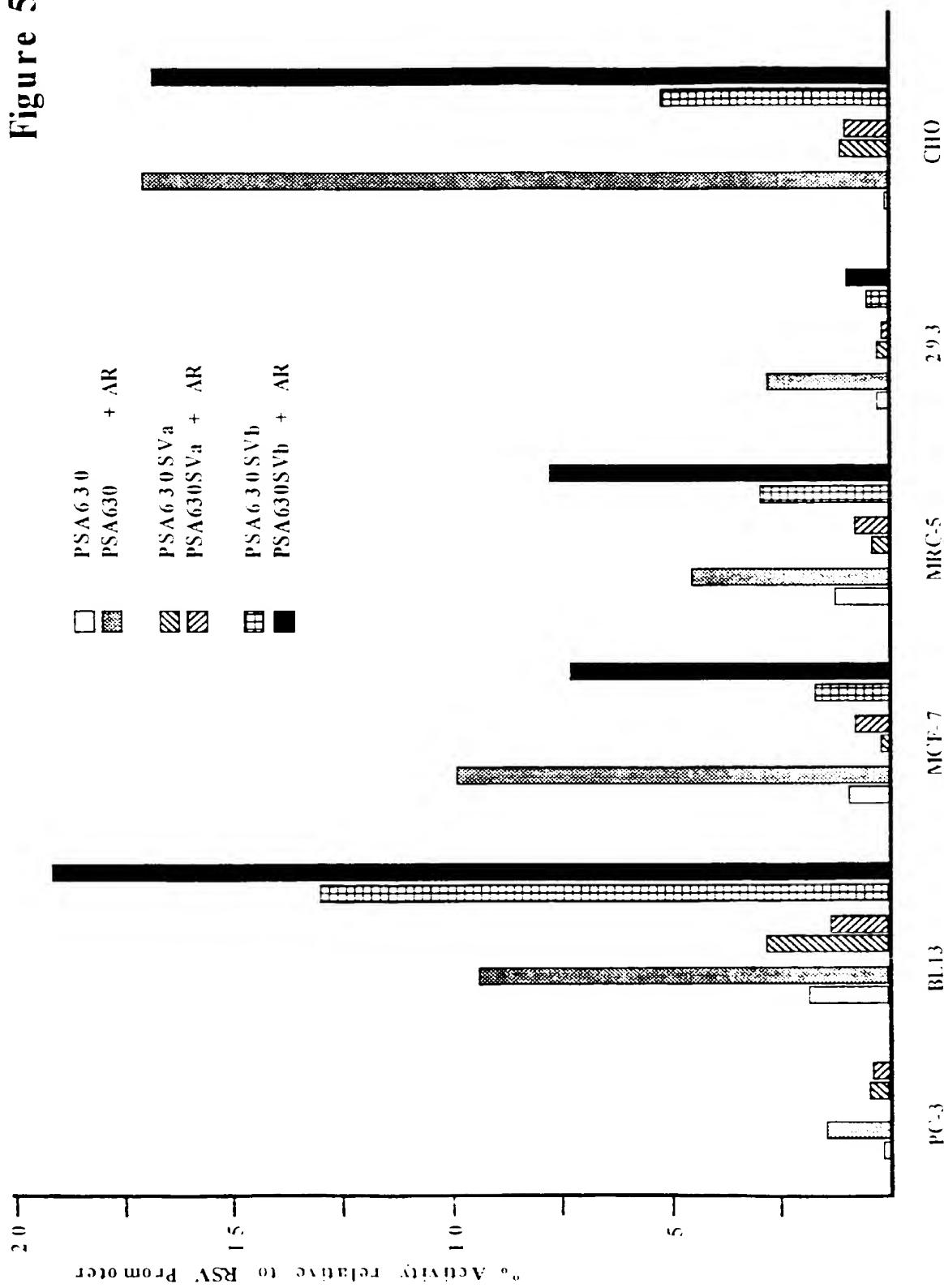


Figure 3

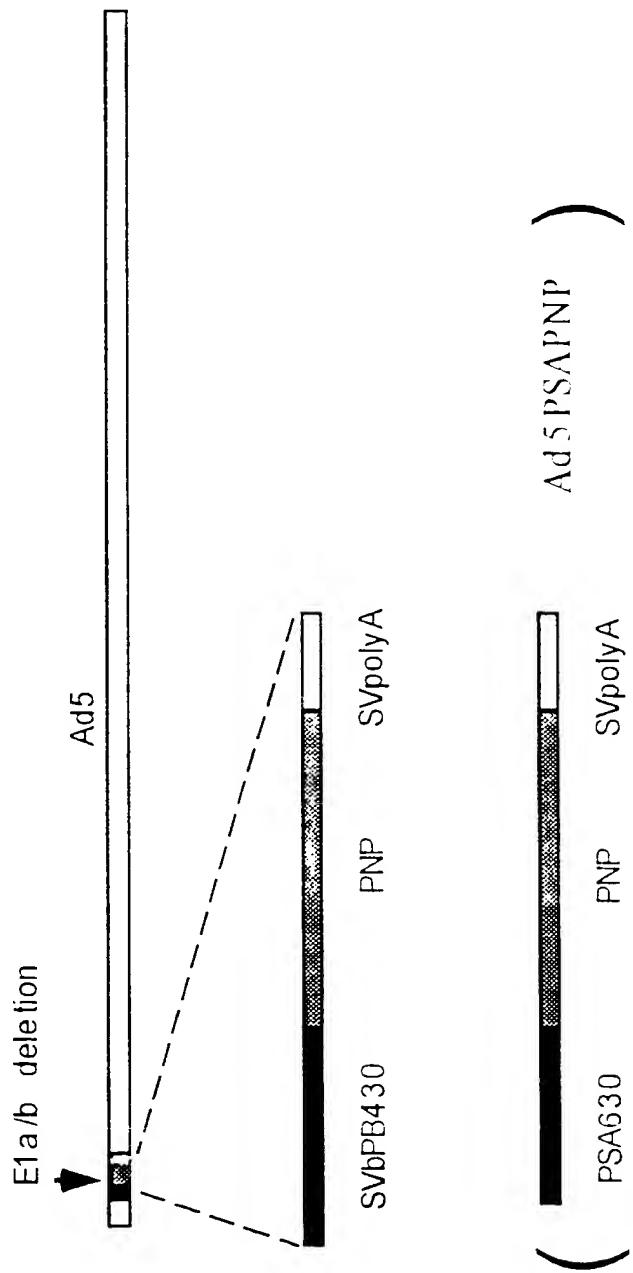
4 / 8

**Figure 4**



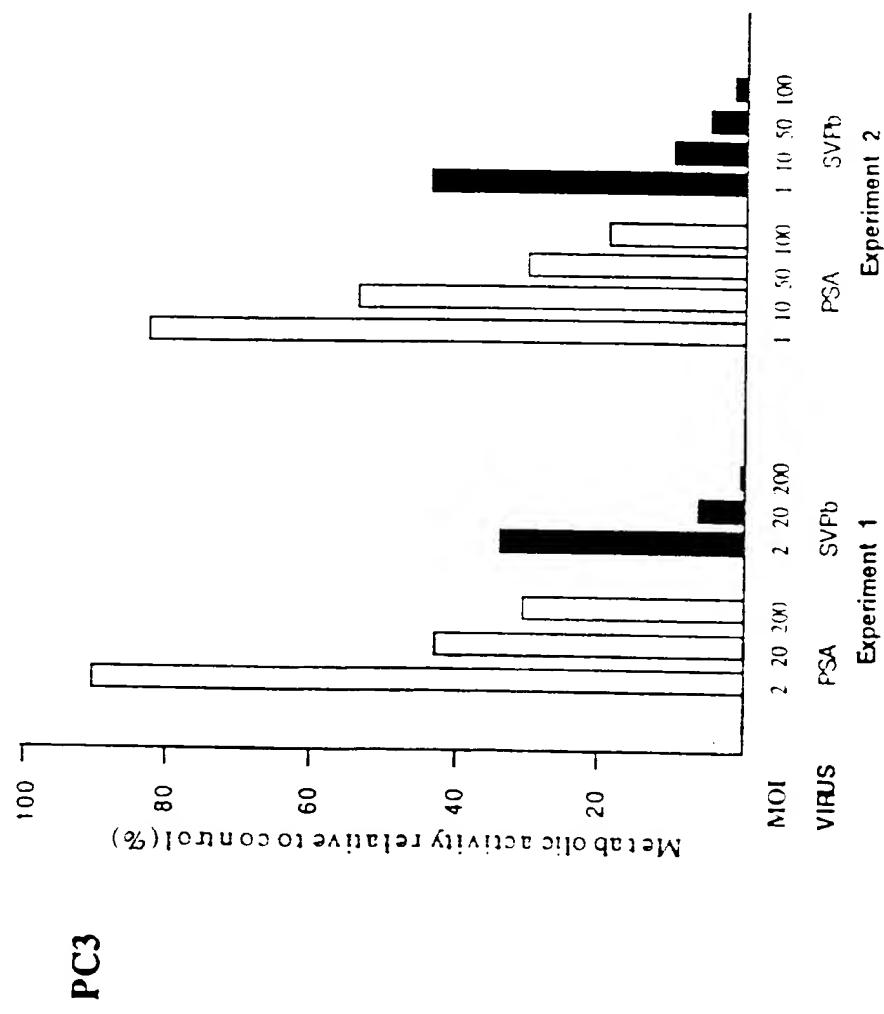
**Figure 5**

**Figure 6.** Ad5SVbPB430PNP

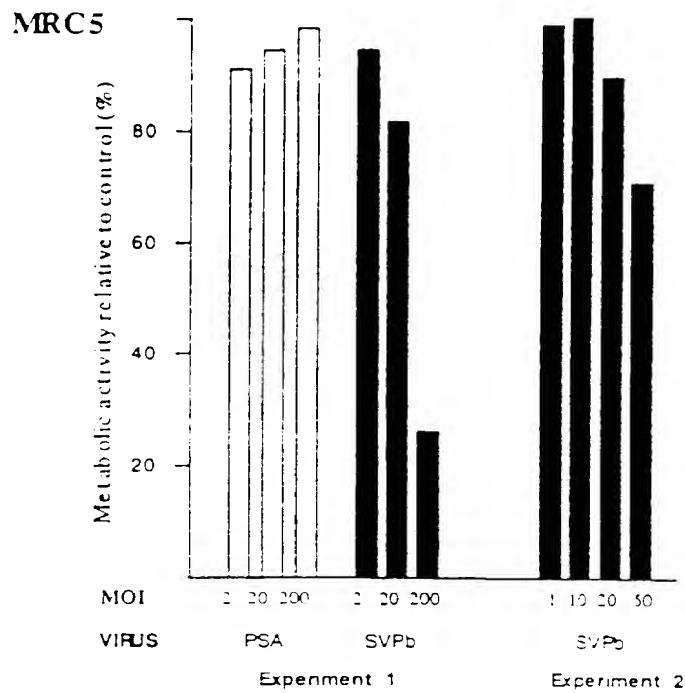
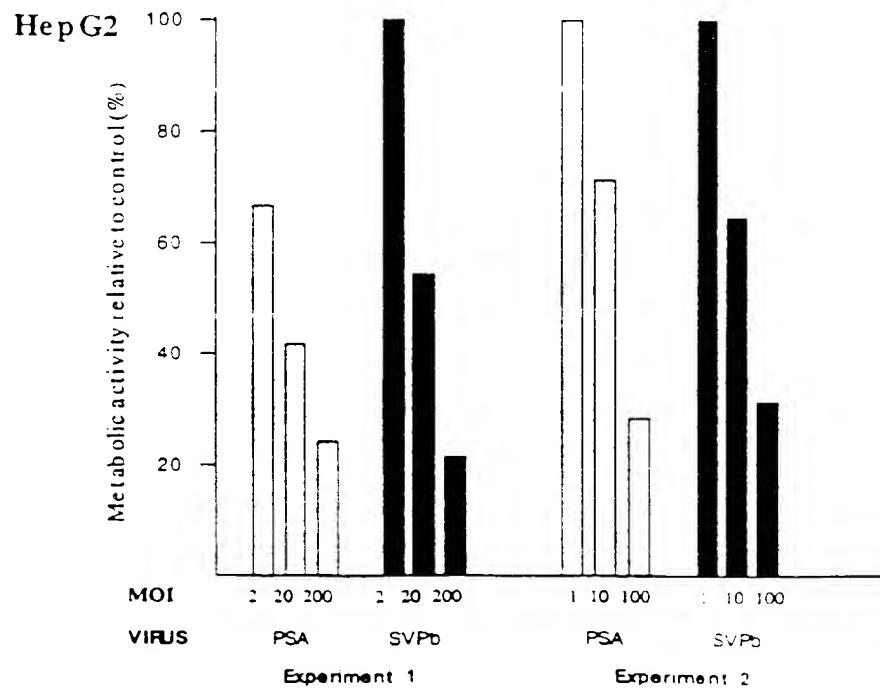


7/8

Figure 7 A

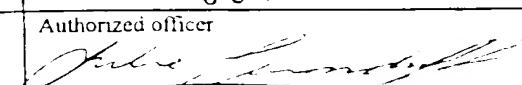


8/8

**FIGURE 7B****FIGURE 7C**

# INTERNATIONAL SEARCH REPORT

International Application No.  
PCT/AU 98/00057

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>		
Int Cl <sup>o</sup> C12N 15/11, 15/62 According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b>		
Minimum documentation searched (classification system followed by classification symbols) IPC6		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched SEE DATABASES BELOW		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) <u>WPAT</u> : steroid#()respon:()promoter#, steroid#()respon:()element#, androgen#()respon:()promoter#, androgen#()respon:()element#, tissue#()specific, prostate()specific, SV40(5n)enhancer, probasin:()promoter#, probasin#, prostate()specific ()antigen#, PSA <u>MEDLINE</u> : SV40, enhancer, steroid, hormone, androgen, prostate <u>CHEMICAL ABSTRACTS</u> : SV40, enhancer, steroid, hormone, androgen, prostate, prostate specific antigen		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	BRITISH JOURNAL OF UROLOGY (1997 March) volume 79, supplement 1, pages 97-103, Dannull J and Belldegrum AS "Development of gene therapy for prostate cancer using a novel promoter of prostate specific antigen". See entire document	1-21
X	WO 96/14875 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 23 May 1996. See entire document	1-21
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C		<input type="checkbox"/> See patent family annex
<ul style="list-style-type: none"> <li>* Special categories of cited documents:</li> <li>"A" document defining the general state of the art which is not considered to be of particular relevance</li> <li>"E" earlier document but published on or after the international filing date</li> <li>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</li> <li>"O" document referring to an oral disclosure, use, exhibition or other means</li> <li>"P" document published prior to the international filing date but later than the priority date claimed</li> </ul>		
Date of the actual completion of the international search 25 March 1998	Date of mailing of the international search report 08 APR 1998	
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 209 WODEN ACT 2606 AUSTRALIA	Authorized officer  <b>JULIE CAIRNDUFF</b> Telephone No. +61 2 6283 2747	
Faximile No. +61 2 6285 3929		

**INTERNATIONAL SEARCH REPORT**

International Application No.

PCT/AU 98/00057

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT	
Category*	Citation of document, with indication, where appropriate, of the relevant passages
A	CANCER RESEARCH, (1997, February 1) volume 57, pages 495-499, Pang S. et al. "Identification of a positive regulatory element responsible for tissue-specific expression of prostate-specific antigen" See entire document